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519491

PENICILLIN DERIVATIVES AND PROCESS FOR PREPARATION OF THE SAME

This invention relates to penicillin derivatives and to a process for preparing them.

Of the commercially available antibiotics, ß-lactam type antibiotics having a ß-lactam ring, namely penicillins and cephalosporins, are best known and frequently used. Although widely used as useful chemotherapeutic drugs, the ß-lactam type antibiotics can not achieve satisfactory effects against some types of microorganisms because of resistance of the microorganism to the ß-lactam type antibiotics. The resistance thereof are usually attributable to ß-lactamase produced by the microorganism. The ß-lactamase is an enzyme which acts to cleave the ß-lactam ring of the ß-lactam type antibiotic, thereby causing the antibiotic to lose its antimicrobial activity. For this reason, the action of ß-lactamase must be eliminated or inhibited so as to enable the ß-lactam type antibiotic to produce satisfactory effects. The elimination or inhibition of the ß-lactamase activity can be achieved by ß-lactamase inhibitors, which are used conjointly with the ß-lactam type antibiotic to increase the antimicrobial activity of the antibiotic.

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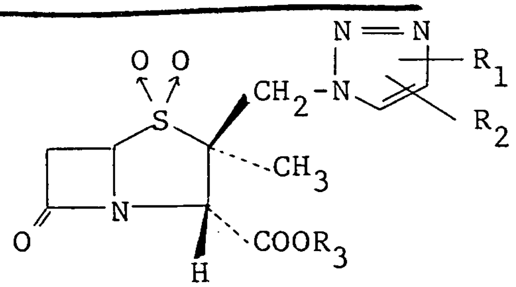
It is an object of the present invention to provide novel compounds having ß-lactamase inhibitory action.

It is another object of the invention to provide processes for preparing the same.

It is a further object of the invention to provide a pharmaceutical composition having excellent ß-lactamase inhibitory action.

It is an additional object of the invention to provide compositions which, when combined with ß-lactam type antibiotics, can increase the antibacterial activity of the antibiotics.

The penicillin derivatives of the present invention are represented by the formula



(I)

wherein R_1 is hydrogen or trialkylsilyl, R_2 is hydrogen, trialkylsilyl or $COOR_2'$ wherein R_2' is hydrogen, C_{1-18} alkyl, C_{2-7} alkoxymethyl, C_{3-8} alkylcarbonyloxymethyl, C_{4-9} alkylcarbonyloxyethyl, (C_{5-7} cycloalkyl)carbonyloxymethyl, C_{9-14} benzylcarbonyloxyalkyl, C_{3-8} alkoxycarbonylmethyl, C_{4-9} alkoxycarbonylethyl, phthalidyl,

65 crotonolacton-4-yl, γ-butyrolacton-4-yl, halogenated C_{1-6} alkyl substituted with 1 to 3 halogen atoms, C_{1-6} alkoxy- or nitro-substituted or unsubstituted benzyl, benzhydryl, tetrahydropyranyl, dimethylaminoethyl, dimethylchlorosilyl, trichlorosilyl, (5-substituted C_{1-6} alkyl or phenyl or unsubstituted-2-oxo-1,3-dioxoden-4-yl)methyl, C_{8-13} benzoyloxyalkyl and group for forming a pharmaceutically acceptable salt; and R_3 has the same 40 meaning as R_2'.

62 The penicillin derivatives of the present invention are all novel compounds and have ß-lactamase inhibitory properties, hence useful as ß-lactamase inhibitory agents.

62 The penicillin derivatives of the invention, when used in combination with a known ß-lactam type antibiotic, can increase the antimicrobial activity 62 of the ß-lactam type antibiotic.

Examples of antibiotics which can be used conjointly with the compounds of the present invention are ß-lactam antibiotics which exhibit antibacterial action against gram-positive or gram-negative bacteria and which include commonly used penicillins such as ampicillin, amoxicillin, hetacillin, ciclacillin, mecillinam, carbenicillin, sulbenicillin, ticarcillin, piperacillin, apalcillin, methicillin, mezlocillin

and salts thereof; esters of penicillins such as bacampicillin, carindacillin, talampicillin, carfecillin and pivmecillinam; cephalosporins such as cephaloridine, cephalothin, cephapirin, cephacetrile, cefazolin, cephalexin, cefradine, cefotiam, cefamandole, cefuroxime, cefoxitin, cefmetazole, cefsulodin, cefoperazone, cefotaxime, ceftizoxime, cefmenoxime, latamoxef, cefaclor, cefroxadine, cefatrizine, cefadroxil, cephaloglycin, and salts thereof. The ß-lactam antibiotics are usually used in an amount of about 0.1 to about 10 parts by weight, preferably about 0.2 to about 5 parts by weight, per part by weight of the compound of the invention.

Examples of the trialkylsilyl groups represented by R_1 and R_2 in the formula (I) include trialkylsilyl having straight-chain or branched-chain C_{1-6} alkyl such as methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl, hexyl, and the like.

Examples of the group R_2' of $COOR_2'$ represented by R_2 in the formula (I) include; C_{1-18} alkyl such as methyl, ethyl, propyl, isopropyl, tert-butyl, pentyl, hexyl, decyl, undecyl, dodecyl, tetradecyl, hexadecyl, octadecyl and like straight- or branched-chain alkyl; C_{2-7} alkoxymethyl such as methoxymethyl, ethoxymethyl, propyloxymethyl, isopropyloxymethyl, butoxymethyl and hexyloxymethyl; C_{3-8} alkylcarbonyloxymethyl such as

methylcarbonyloxymethyl, ethylcarbonyloxymethyl, butylcarbonyloxymethyl and hexylcarbonyloxymethyl; C_{4-9} alkylcarbonyloxyethyl such as methylcarbonyloxyethyl, ethylcarbonyloxyethyl, butylcarbonyloxyethyl and pivaloyloxyethyl; (C_{5-7} cycloalkyl)carbonyloxymethyl such as cyclopentylcarbonyloxymethyl, cyclohexylcarbonyloxymethyl and cycloheptylcarbonyloxymethyl; C_{9-14} benzylcarbonyloxyalkyl such as benzylcarbonyloxymethyl, benzylcarbonyloxyethyl, benzylcarbonyloxypropyl and benzylcarbonyloxybutyl; C_{3-8} alkoxycarbonylmethyl such as methoxycarbonylmethyl, ethoxycarbonylmethyl, propyloxycarbonylmethyl and hexyloxycarbonylmethyl; C_{4-9} alkoxycarbonylethyl such as methoxycarbonylethyl, ethoxycarbonylethyl, propyloxycarbonylethyl, butoxycarbonylethyl and hexyloxycarbonylethyl; halogenated C_{1-6} alkyl substituted with 1 to 3 halogen atoms such as chloromethyl, 2,2-dibromoethyl and trichloroethyl; C_{1-6} alkoxy- or nitro-substituted or unsubstituted benzyl such as p-methoxybenzyl, p-ethoxybenzyl, o-nitrobenzyl and p-nitrobenzyl; (5-substituted C_{1-6} alkyl or phenyl or unsubstituted-2-oxo-1,3-dioxoden-4-yl)methyl such as (2-oxo-1,3-dioxoden-4-yl)methyl, (5-methyl-2-oxo-1,3-dioxoden-4-yl)methyl and (5-phenyl-2-oxo-1,3-dioxoden-4-yl)methyl; C_{8-13} benzoyloxyalkyl such as benzoyloxymethyl, benzoyloxyethyl, benzoyloxy-

propyl and benzoyloxybutyl; etc.

Examples of the groups represented by R_3 in the formula (I) are the same as those exemplified in respect of the group R_2'.

The ester residues represented by R_2' and R_3 include both carboxyl-protecting groups acceptable in the synthesis of penicillin compounds and pharmaceutically acceptable ester residues. A pharmaceutically acceptable ester having such residue is an ester which is easily hydrolyzed in vivo and which is a non-poisonous ester capable of rapidly decomposing in the blood or tissue of humans, thereby producing the corresponding acid of the formula (I) in which R_3 is hydrogen atom. Generally in the synthesis of penicillin compounds, ester-protecting groups are used in the art to protect penicillin carboxyl groups or other carboxyl groups. While it is difficult to determine which ester-protecting group should be used, consideration are usually given to select esters in which the protecting group per se is sufficiently stable in the reaction and which does not permit cleavage of the ß-lactam ring in removal of the ester-protecting groups. Most commonly used as such ester-protecting groups are p-nitrobenzyl group, benzhydryl group, trichloroethyl group, trichlorosilyl group, tetrahydropyranyl group, etc. Examples of the pharmaceutically acceptable ester

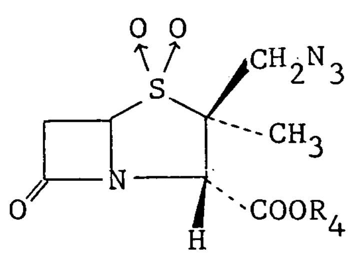
65 groups are phthalidyl, crotonolacton-4-yl, γ-butyrolacton-4-yl, (2-oxo-1,3-dioxoden-4-yl)methyl, etc.

40 Examples of the group for forming a pharmaceutically acceptable salt represented by R_2' and R_3 in the formula (I) include; sodium, potassium, lithium, or like alkali metal atoms; calcium, magnesium or like alkaline earth metal atoms; cyclohexylamine, trimethylamine, diethanolamine or like ~~the~~ organic amine ~~residues~~; ~~alginine~~ arginine, lysine or like basic amino acid residues; ammonium residues, etc.

The penicillin derivatives of the present invention having the formula (I) can be prepared by the processes as shown in reaction equations given below. The processes differ according to the kind of the groups represented by R_1 and R_2.

Reaction Equation-1

T80X



(II)

$$\xrightarrow[\text{Step (A)}]{R_1C \equiv CR_5 \quad (III)}$$

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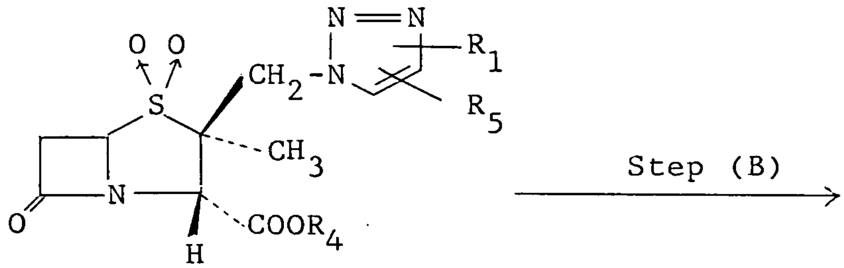
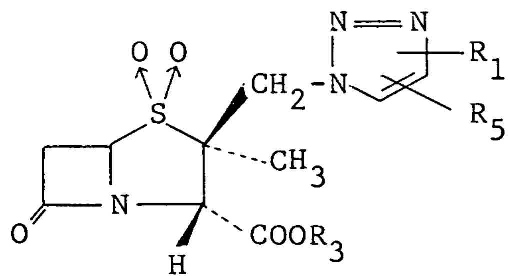


(IV)



(I-a)

In the foregoing formulae, R_1 and R_3 are as defined above, R_4 is penicillin carboxyl-protecting group and R_5 is trialkylsilyl or $COOR_2'$ wherein R_2' is as defined above.

Examples of the penicillin carboxyl protecting group expressed by R_4 include known groups such as those described in Japanese Unexamined Patent Publication No.81380/1974 and H.E. Flynn, "Cephalosporins and Penicillins, Chemistry and Biology" (published in 1972 by Academic Press). Specific examples thereof are ethyl, propyl, tert-butyl, trichloroethyl and like substituted or unsubstituted alkyl groups; benzyl,

diphenyl methyl, p-nitrobenzyl and like substituted or unsubstituted aralkyl groups; acetoxymethyl, acetoxyethyl, propionyloxyethyl, pivaloyloxyethyl, pivaloyloxypropyl, benzyloxymethyl, benzyloxyethyl, benzylcarbonyloxymethyl, cyclohexylcarbonyloxymethyl and like acyloxyalkyl groups, methoxymethyl, ethoxymethyl, benzyloxymethyl and like alkoxyalkyl groups; and other groups such as tetrahydropyranyl, dimethylaminoethyl, dimethylchlorosilyl, trichlorosilyl and like groups.

The steps (A) and (B) of the foregoing process will be described below in detail.

CL <u>Step (A)</u>

P A penicillanic acid derivative of the formula (II) is reacted with an acetylene derivative of the formula (III) to provide a compound of the formula (IV). The reaction is conducted in a suitable solvent by reacting a known penicillanic acid derivative of the formula (II) with a known acetylene derivative of the formula (III) in an amount of about 1 to about 50 moles, preferably about 1 to about 10 moles, per mole of the derivative of the formula (II).

The solvents useful in the reaction are not particularly limited and include any of those which do not adversely affect the reaction. Specific examples

of the solvents are an acetylene derivative of the formula (III) as used in excess amount or benzene, toluene, xylene and like aromatic hydrocarbons, tetrahydrofuran, dioxane or like ethers, acetone and like polar organic solvents; etc. These solvents are used singly or in mixture. The reaction proceeds usually at a temperature of between about 50°C and a boiling point of the solvent, or at a temperature of less than 200°C in a sealed reactor, and goes to completion in about 2 to about 72 hours.

Depending upon the kind of the penicillin carboxyl protecting group represented by R_4, the compounds of the formula (IV) obtained in step (A) may be esters of the penicillin derivatives of the present invention having the formula (I). The compounds of the formula (IV) are preferably subjected to de-esterification to form a derivative of the formula (I-a) in which R_3 is hydrogen which, in turn, is converted into a pharmaceutically acceptable salt or ester thereof as in the following step (B). The compound of the formula (IV) can also be made into an ester of the formula (I-a) by the conventional ester interchange reaction in the step (B).

Step (B)

The compound of the formula (IV) is subjected

to de-esterification without or after isolation from the reaction mixture obtained in step (A), whereby a penicillin derivative of the formula (I-a) in which R_3 is hydrogen is obtained.

As the de-esterification method, reduction, hydrolysis, treatment with an acid and like method can be employed for converting the carboxyl-protecting group to carboxyl group. For example, if the carboxyl-protecting group is an active ester, the reaction frequently proceeds with ease under mild hydrolysis conditions or by merely bringing the ester into contact with water. The reduction method is employed when the carboxyl-protecting group is trichloroethylbenzyl, p-nitrobenzyl, diphenylmethyl or the like. Treatment with an acid is adopted when the carboxyl-protecting group is 4-methoxybenzyl, tert-butyl, trityl, diphenylmethyl, methoxymethyl, tetrahydropyranyl or the like.

The reduction can be conducted by treating the ester of the formula (IV) with a mixture of (a) zinc, zinc-amalgam or like metal and/or chromium chloride, chromium acetate or like chromium salt and (b) formic acid, acetic acid or like acid. Alternatively, the reduction can be conducted with use of a catalyst in hydrogen atomosphere in a solvent. Examples of the catalysts are platinum, platinum oxide, palladium,

palladium oxide, palladium-barium sulfate, palladium-calcium carbonate, palladium-carbon, nickel oxide, Raney-nickel, etc. The solvents are not particularly limited so far as they do not adversely affect the reaction, and include methanol, ethanol and like alcohols; tetrahydrofuran, dioxane and like ethers; ethyl acetate and like esters; acetic acid and like fatty acids; and a mixture of these organic solvents and water.

The acids useful for eliminating the carboxyl-protecting group of the ester of the formula (I-a) are formic acid, acetic acid and like lower fatty acids; trichloroacetic acid, trifluoroacetic acid and like trihalogenated acetic acids; hydrochloric acid, hydrofluoric acid and like hydrohalogenic acids; p-toluene-sulfonic acid, trifluoromethane-sulfonic acid and like organic sulfonic acids; and a mixture of these. In this reaction, when the acid used is in a liquid state and acts also as a solvent, it is not necessary to use other solvents. However, dimethylformamide, dichloromethane, chloroform, tetrahydrofuran, acetone and like solvents which do not adversely affect the reaction may be used.

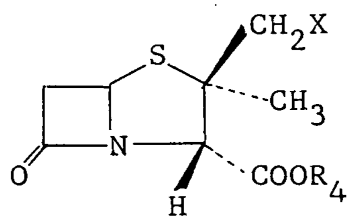
The penicillin derivative of the present invention having the formula (I-a) in which R_3 is

hydrogen can be transformed by the salt-forming reaction or esterification commonly employed in the art into a pharmaceutically acceptable salt or ester as contemplated.

If the ester residue is, for example, 3-phthalidyl, crotonolacton-4-yl, γ-butyrolacton-4-yl or like group, the penicillin derivative of the formula (IV) can be alkylated by using 3-halogenated phthalide, 4-halogenated crotonolactone, 4-halogenated-γ-butyrolactone or the like. Suitable halogens of the foregoing halides include chlorine, bromine, iodine, etc. The reaction is carried out by dissolving the salt of the penicillin derivative of the formula (IV) in N,N-dimethylformamide or like suitable polar organic solvent and adding an approximately equimolecular amount of a halide to the solution. The reaction temperature ranges from about 0 to about 100°C, preferably from about 15 to about 35°C. Suitable salts of the penicillin derivative to be used in the esterification are salts of sodium, potassium or like alkali metals; salts of triethylamine, ethyldiisopropylamine, N-ethylpiperidine, N,N-dimethylaniline, N-methylmorpholine or like tertiary amines, etc. After completion of the reaction, the contemplated product can be easily separated by the conventional method and also can be purified, when required, by recrystallization, thin layer chromatography, column chromatography or like method.

The compound of the formula (II) to be used as the starting material in the step (A) is a novel compound undisclosed in literature and can be synthesized by the method described in Japanese Patent Application No.69142/1982 (relating to an invention accomplished by us). The disclosed method comprises the steps of reacting a metal azide with a known derivative of penicillanic acid of the formula

T150x



(V)

PS

wherein X represents chlorine atom or bromine atom and R_4 is as defined above, oxydizing the reaction mixture and subjecting the resulting compound to de-esterification.

The foregoing method will be described below in detail. The reaction between the compound of the formula (V) and the metal azide is conducted in a suitable solvent by using the metal azide in an amount of about 1 to about 50 moles, preferably about 1 to about 10 moles, per mole of the compound of the formula (V). Examples of the metal azides which can be used include those commonly used, such as sodium azide, potassium azide and like azides of alkali metals, and

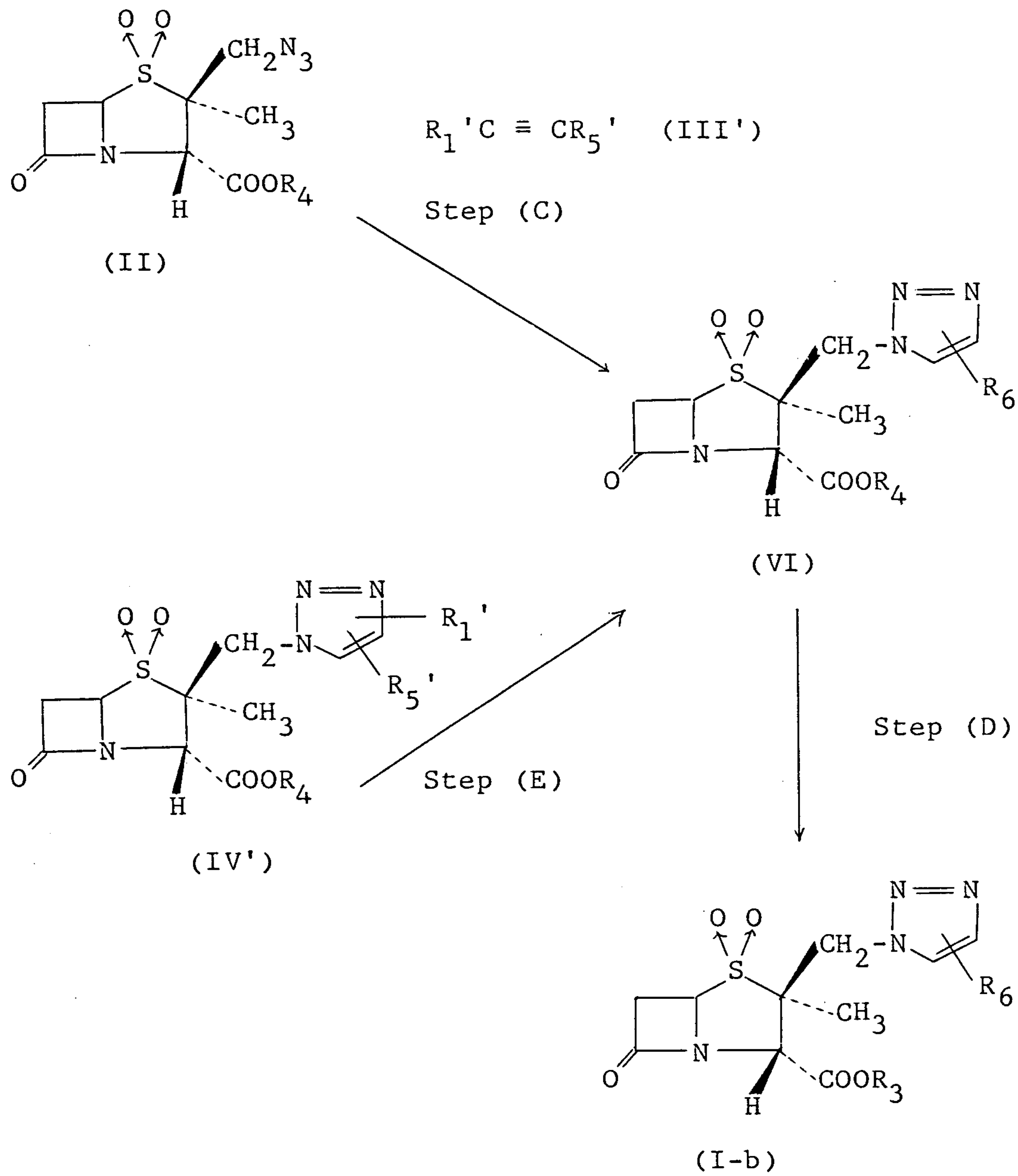
barium azide and like azides of alkaline earth metals. Useful solvents are not particularly limited as far as they do not adversely affect the reaction. Examples of useful solvents are dimethylformamide, ethyl acetate, acetone, dichloromethane, tetrahydrofuran, dioxane, methanol, ethanol and like organic solvents. These organic solvents can be used singly or in mixtures. Also a mixture of such solvent and water is usable. The reaction proceeds at a temperature of usually about -20 to about 100°C, preferably about 0 to about 100°C. The resulting product can be used in subsequent oxidation without isolation, or alternatively after isolation and purification by a conventional method. The oxidation subsequent to the azide-forming reaction is conducted by using an oxidizing agent commonly employed such as permanganic acid, periodic acid, peracetic acid, performic acid, trifluoroperacetic acid, perbenzoic acid, m-chloroperbenzoic acid, hydrogen peroxide, etc. The oxidizing agent can be used in large excess, and may be employed preferably in an amount of about 1 to about 2 moles per mole of the starting compound. The oxidation is carried out usually in a suitable solvent. Useful solvents include any of those which do not adversely affect the oxidation reaction such as chloroform, pyridine, tetrahydrofuran, dioxane, methylene chloride,

carbon tetrachloride, acetic acid, formic acid, dimethylformamide, water, etc. The oxidation is performed at a temperature which is not particularly limited but generally ranges from room temperature to cooling temperature, preferably about 0 to about 30°C.

The compound thus obtained is subjected to de-esterification whereby the compound of the formula (II) can be produced. The de-esterification is effected under the same conditions as shown in the reaction scheme of the step (B). The process for preparing the compound of the formula (II) is described in detail in reference examples to be set forth later.

Reaction Equation-2

T180X



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In the foregoing formulae, R_4 is as defined above, R_1' and R_5' are the same groups as those represented by R_1 and R_5 and at least one of them is trialkylsilyl group, and R_6 represents hydrogen or $COOR_2'$ wherein R_2' is as defined above.

The compound of the formula (I) wherein at least one of R_1 and R_2 is hydrogen atom, namely the compound of the formula (I-b), can be prepared by the process shown above in Reaction Equation-2. The steps in the process are set forth below in detail.

Step (C)

The compound of the formula (II) is reacted with a compound of the formula (III') in a solvent such as dichloromethane, dichloroethane, chloroform or like halogenated hydrocarbons. During this reaction, reaction for removing the trialkylsilyl group proceeds at the same time, whereby a compound of the formula (VI) is produced. Useful solvents are not particularly limited as far as they are halogenated hydrocarbons. The reaction conditions including the reaction temperature, the proportions of the reagents to be used and the reaction time are similar to those in the step (A).

Depending upon the kind of the penicillin carboxyl-protecting group represented by R_4, the compound of the formula (VI) thus obtained may be the product as

contemplated, i.e., an ester of the penicillin derivative of the formula (I). More preferably the ester of the formula (VI) is subjected to de-esterification as in the step (B) so that the compound is transformed to a penicillin derivative of the present invention having the formula (I-b) in which R_3 is hydrogen which is converted, when required, in the conventional manner into a pharmaceutically acceptable salt thereof or ester thereof as contemplated.

<u>Step (D)</u>

The compound of the formula (VI) is subjected to de-esterification after or without isolation from the reaction product obtained in the step (C), whereby a penicillin derivative of the formula (I-b) in which R_3 is hydrogen is produced. The de-esterification is carried out under the same conditions as those described above in respect of the step (B).

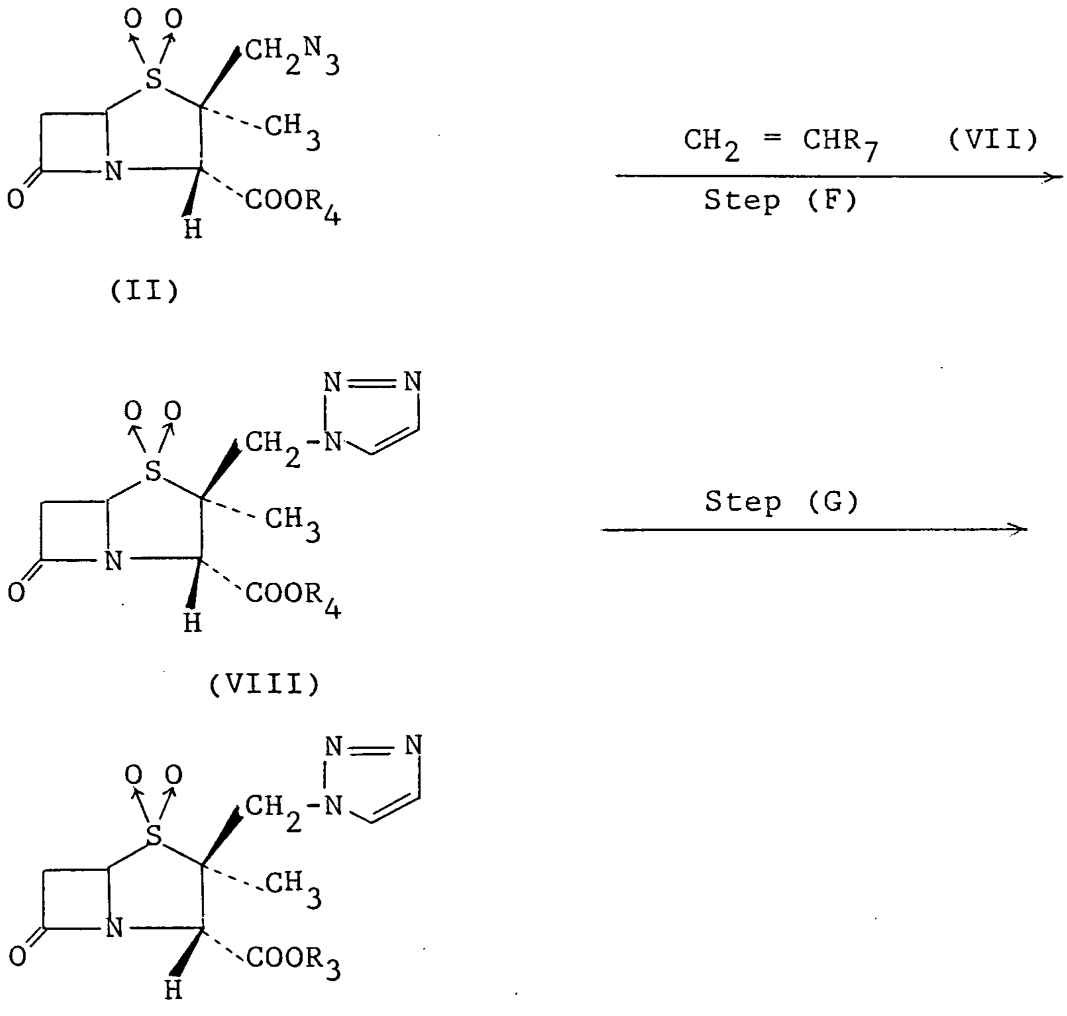
The compound of the formula (VI) can be prepared by the process in the step (C) and also by the process to be set forth below in a step (E).

<u>Step (E)</u>

The compound of the formula (IV) obtained in the step (A) as shown in Reaction Equation-1 wherein at least one of R_1 and R_5 is trialkylsilyl, namely the compound of the formula (IV'), is subjected to reaction for

removing the trialkylsilyl in the presence of potassium fluoride after or without isolation from the reaction product obtained in the step (A), whereby a compound of the formula (VI) is produced. The trialkylsilyl removing reaction is conducted in a suitable solvent by using potassium fluoride in an amount of over about 1 mole, preferably about 1 mole, and a catalyst in an amount of about 1/50 to about 1/10 mole, both per mole of the compound of the formula (IV). Useful as the catalyst is a phase transfer catalyst such as quaternary ammonium salt, crown ether or the like. Examples of useful solvents are any suitable solvents which do not adversely affect the reaction and which include benzene, toluene, xylene or like aromatic hydrocarbons; acetonitrile, N,N-dimethylformamide, dimethylsulfoxide or like non-protonic polar solvents; etc. The reaction temperature and reaction time are appropriately determined. Generally the reaction is performed at a temperature in the range of room temperature to about 100°C, and completes in about 1 to about 10 hours.

Reaction Equation-3



(I-c)

In the foregoing formulae, R_4 is as defined above, and R_7 represents acyloxy group.

Examples of the acyloxy groups represented by R_7 are lower acyloxy groups having 2 to 5 carbon atoms such as acetoxy, propionyloxy, butyryloxy, valeryloxy or like aliphatic acyloxy groups and benzoyloxy or like

aromatic acyloxy groups, etc.

The compound of the formula (I) wherein R_1 and R_2 are hydrogen atoms, namely the compound of the formula (I-c), can be produced by the process as shown above in Reaction Equation-3.

The steps (F) and (G) in Reaction Equation-3 will be described below in detail.

CL

Step (F)

P

The penicillanic acid derivative of the formula (II) is reacted with a vinyl derivative of the formula (VII) while reaction for removing the acyloxy group represented by R_7 in the formula (VII) is carried out, whereby a compound of the formula (VIII) is prepared. The reaction between the penicillanic acid derivative of the formula (II) and the vinyl derivative of the formula (VII) is conducted in the presence of or in the absence of a suitable solvent by using the vinyl derivative of the formula (VII) in an amount of at least about 1 mole, preferably about 1 to about 200 moles, per mole of the derivative of the formula (II), whereby there occurs simultaneously the acyloxy-removing reaction. The solvents which can be used are not particularly limited as far as they do not adversely affect the reaction. Specific examples thereof are benzene, toluene, xylene or like aromatic hydrocarbons, tetra-

hydrofuran, dioxane or like ethers, etc. The reaction is effected at a temperature ranging from about 50°C to a boiling point of the solvent, or a temperature of less than 200°C in a sealed reactor, and is completed in about 2 to about 72 hours. Depending on the kind of the penicillin carboxyl-protecting group represented by R_4 in the formula (VIII), the compound of the formula (VIII) thus obtained may be the product as contemplated, namely the ester of the penicillin derivative of the formula (I). More preferably the compound of the formula (VIII) thus prepared is subjected to de-esterification as in the step (G) so that the compound is converted by the conventional method into a penicillin derivative of the formula (I-c) wherein R_3 is hydrogen which, in turn, is transformed by the conventional method into a pharmaceutically acceptable salt thereof or ester thereof as contemplated. The compound of the formula (VIII) can be made into a pharmaceutically acceptable salt thereof or ester thereof as contemplated by conducting an ester interchange or salt-forming reaction in the conventional manner.

Step (G)

The compound of the formula (VIII) is subjected to de-esterification after or without isolation from the reaction product obtained in the step (F), whereby

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a penicillin derivative of the formula (I-c) in which R_3 is hydrogen is produced. The reaction conditions for de-esterification are the same as those described in the step (B).

After completion of the reaction in each step, the contemplated compound producible in each step can be isolated from the reaction product or, when required, can be purified by the conventional method such as recrystallization method, thin-layer chromatography, column chromatography or the like.

The penicillin derivative of the present invention is mixed with the ß-lactam type antibiotic substance to form a preparation which is orally or parenterally administered. Alternatively, the present compound and a suitable antibiotic can be separately administered. Thus the derivatives of the formula (I) can be used for treating infectious disease of human beings and other animals.

The composition of the present invention may be made into tablets, pills, capsules, granules, powders, syrups, lozenges, solutions, suspensions, etc. for oral administration and aqueous, suspending or water-soluble preparations for intravenous, subcutaneous or intramuscular injections.

Carriers useful in formulating the preparations

are commonly used pharmaceutically acceptable non-toxic carriers such as gelatin, lactose, starch, magnesium stearate, talc, vegetable oil, animal oil, polyalkylene glycol, etc. The carrier may be used with other additives such as diluents, binders, buffer agents, preservatives, glazes, disintegrators, coating agents, etc.

The daily dose of the preparation can be appropriately determined and is not particularly limited. Preferably the daily dose is such that the total amount of the present compound and ß-lactam antibiotic is about 1 to about 200 mg/Kg body weight for oral administration and about 1 to about 100 mg/Kg body weight for parenteral administration.

The present invention will be described below in more detail with reference to examples given below.

Reference Example 1

Preparation of benzhydryl 2ß-azidomethyl-2α-methylpenam-3α-carboxylate

A solution of 5.00 g of sodium azide in 53 ml of water was added to a solution of benzhydryl 2ß-chloromethyl-2α-methylpenam-3α-carboxylate (5.13 g) in dimethylformamide (155 ml). The mixture was stirred at room temperature for 4 hours. The resulting reaction mixture was poured into cooled water and the mixture was extracted with ethyl acetate. The ethyl acetate layer was washed

with water, dried over magnesium sulfate and concentrated to provide 4.87 g of the contemplated product as oil in 93 % yield.

Infrared absorption spectrum (nujol)

νmax (cm^{-1}): 2120, 1812, 1765

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.30 (3H, s), 3.25 (2H, m), 3.42 (1H, d), 3.63 (1H, d), 4.75 (1H, s), 4.76 (1H, m), 7.00 (1H, s), 7.40 (10H, s)

Reference Example 2

Preparation of benzhydryl 2ß-azidomethyl-2α-methylpenam-3α-carboxylate 1,1-dioxide

To a solution of benzhydryl 2ß-azidomethyl-2α-methylpenam-3α-carboxylate (7.03 g) in a mixture of acetic acid (240 ml) and water (40 ml) was added potassium permanganate (6.02 g) over a period of more than 1 hour. The mixture was stirred at room temperature for 2.5 hours. The resulting reaction mixture was diluted with ice water. The precipitate was collected by filtration, and washed with water. The resulting product was dissolved in ethyl acetate and the solution was washed with an aqueous solution of sodium hydrogencarbonate and dried over magnesium sulfate. Concentration gave 5.48 g of the contemplated product in 72 % yield.

Infrared absorption spectrum (nujol)

νmax (cm^{-1}): 2120, 1812, 1765

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.18 (3H, s), 3.50 (2H, d), 3.72 (1H, d), 3.93 (1H, d), 4.60 (1H, m), 4.65 (1H, s), 7.00 (1H, s), 7.36 (10H, s)

Reference Example 3

Preparation of p-nitrobenzyl 2ß-azidomethyl-2α-methylpenam-3α-carboxylate

The procedure of Reference Example 1 was repeated with the exception of using as the starting material p-nitrobenzyl 2ß-chloromethyl-2α-methylpenam-3α-carboxylate, affording the above contemplated compound.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 2120, 1798, 1760

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.40 (3H, s), 3.12 (1H, dd), 3.50 (2H, s), 3.62 (1H, dd), 4.83 (1H, s), 5.29 (2H, s), 5.36 (1H, dd), 7.56 (2H, d), 8.26 (2H, d)

Reference Example 4

Preparation of p-nitrobenzyl 2ß-azidomethyl-2α-methylpenam-3α-carboxylate-1,1-dioxide

The procedure of Reference Example 2 was followed with the exception of using as the starting material p-nitrobenzyl 2ß-azidomethyl-2α-methylpenam-3α-carboxylate, giving the above contemplated compound.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 2120, 1770

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.42 (3H, s), 3.45-3.60 (2H, m), 3.75 (1H, d), 3.96 (1H, d), 4.56-4.75 (1H, m), 4.64 (1H, s), 5.33 (2H, s), 7.56 (2H, d), 8.26 (2H, d)

Example 1

Preparation of p-nitrobenzyl 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 1) and p-nitrobenzyl 2ß-(5-ethoxy-carbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 2)

A 2.1 g quantity of p-nitrobenzyl 2ß-azido-methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide and 0.63 g of ethyl propiolate in 62 ml of benzene were refluxed with stirring under nitrogen atmosphere for 37 hours. The solvent was removed by distillation and the residue was subjected to column chromatography on silica gel to produce as a first eluted product 0.7 g

of p-nitrobenzyl 2ß-(5-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide in amorphous form (Compound 2) in 27 % yield.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1795, 1755, 1727

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.39 (3H, s), 1.39 (3H, t), 3.48-3.60 (2H, m), 4.39 (2H, q), 4.58-4.70 (1H, m), 5.11 (1H, s), 5.14 (1H, d), 5.25 (1H, d), 5.31 (1H, d), 5.56 (1H, d), 7.54 (2H, d), 8.09 (1H, s), 8.25 (2H, d).

There was obtained as a second eluted product 1.6 g of p-nitrobenzyl 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide in amorphous form (Compound 1) in 62 % yield.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1800, 1760 (sh), 1733

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.34 (3H, s), 1.41 (3H, t), 3.50-3.65 (2H, m), 4.42 (2H, q), 4.60-4.75 (2H, m), 5.09 (2H, s), 5.36 (2H, s), 7.59 (2H, d), 8.28 (2H, d), 8.30 (1H, s)

Example 2

Preparation of p-nitrobenzyl 2ß-(4-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 3) and p-nitrobenzyl 2ß-(5-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 4)

The contemplated product was synthesized in the same manner as in Example 1 and eluted by column chromatography on silica gel. There was obtained as a first eluted product p-nitrobenzyl 2ß-(5-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide in amorphous form (Compound 4) in 26 % yield.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1795, 1727

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.39 (3H, s), 3.45-3.60 (2H, m), 3.94 (3H, s), 4.58-4.70 (1H, m), 5.09 (1H, s), 5.10-5.64 (4H, m), 7.54 (2H, d), 8.10 (1H, s), 8.25 (2H, d).

There was obtained as a second eluted product p-nitrobenzyl 2ß-(4-methoxycarbonyl-1,2,3-triazol-1-yl)-methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide in amorphous form (Compound 3) in 61 % yield.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1798, 1730

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.33 (3H, s), 3.48-3.68 (2H, m), 3.96 (3H, s), 4.56-4.76 (2H, m) 5.09 (2H, s), 5.36 (2H, s), 7.60 (2H, d), 8.28 (2H, d), 8.30 (1H, s).

Example 3

Preparation of benzhydryl 2ß-(4-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 5) and benzhydryl 2ß-(5-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 6)

The contemplated product was synthesized in the same manner as in Example 1 and eluted by column chromatography on silica gel. First there was eluted benzhydryl 2ß-(5-methoxycarbonyl-1,2,3-triazol-1-yl)-methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 6) in 18 % yield.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1800, 1727

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.20 (3H, s), 3.44-3.58 (2H, m), 3.91 (3H, s), 4.50-4.65 (1H, m),

5.24 (1H, d), 5.25 (1H, s), 5.45 (1H, d), 6.91 (1H, s), 7.20-7.40 (10H, m), 8.08 (1H, s).

Secondly there was eluted benzhydryl 2ß-(4-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (compound 5) in 60 % yield.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1803, 1727

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.05 (3H, s), 3.48-3.62 (2H, m), 3.95 (3H, s), 4.55-4.75 (2H, m), 5.11 (2H, bs), 7.02 (1H, s), 7.20-7.50 (10H, m), 8.25 (1H, s).

Example 4

Preparation of sodium 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 7)

Hydrogenation was conducted at a low pressure and at room temperature by using 15 ml of ethyl acetate, 15 ml of water, 340 mg of p-nitrobenzyl 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide, 60 mg of 10 % palladium charcoal and 110 mg of sodium hydrogencarbonate. After completion of absorption of hydrogen, the reaction mixture was

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filtered to separate the aqueous layer which was washed with benzene. The aqueous solution was concentrated at reduced pressure and the concentrate was subjected to column chromatography using an MCI gel, CHP-20 P (product of Mitsubishi Kasei Co., Ltd., Japan) to conduct gradient elution with a water-10 % acetone water mixture. The eluate thus obtained was freeze-dried to afford 200 mg of the contemplated product (Compound 7) as white powder in 76 % yield. The white powder decomposed at a temperature of more than 180°C.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1782, 1720

Nuclear magnetic resonance spectrum (D_2O)

δ (ppm) : 1.39 (3H, t), 1.46 (3H, s), 3.45 (1H, dd), 3.72 (1H, dd), 4.44 (2H, q), 4.50 (1H, s), 4.96-5.10 (1H, m), 5.18 (1H, d), 5.42 (1H, d), 8.72 (1H, s)

Example 5

Preparation of 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)-methyl-2α-methylpenam-3α-carboxylic acid-1,1-dioxide (Compound 8)

Hydrogenation was conducted at room temperature and at a pressure of 3 atm. by using 4.2 g of p-nitrobenzyl 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-

2α-methylpenam-3α-carboxylate-1,1-dioxide, 1.4 g of sodium hydrogencarbonate, 800 mg of 10 % palladium charcoal, 100 ml of ethyl acetate and 100 ml of water. After completion of absorption of hydrogen, the reaction mixture was filtered and the aqueous layer was separated and washed with benzene. The pH of the aqueous layer was adjusted to 1 to 2 with hydrochloric acid. The aqueous layer was extracted with ethyl acetate and the extract was dried over magnesium sulfate. The solvent was distilled off and 3.0 g of the contemplated compound was produced in amorphous form in 97 % yield.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1798, 1726

Nuclear magnetic resonance spectrum ($DMSO-d_6$)

δ (ppm) : 1.31 (3H, t), 1.42 (3H, s), 3.31 (1H, dd), 3.73 (1H, dd), 4.32 (2H, q), 4.75-5.38 (4H, m), 8.76 (1H, s)

Example 6

Preparation of chloromethyl 2β-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 9)

A 2.2 g quantity of sodium hydrogencarbonate and 0.2 g of tetrabutylammonium hydrogensulfate were added with stirring at a temperature of less than 10°C

to 2.4 g of 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)-methyl-2α-methylpenam-3α-carboxylic acid-1,1-dioxide, 13.5 ml of dichloromethane and 13.5 ml of water. To the mixture was dropwise added at the same temperature 1.25 g of chloromethyl chlorosulfonate and the resulting mixture was stirred at room temperature for 30 minutes. The organic layer was separated, washed once with water and dried over magnesium sulfate. The solvent was removed by distillation and the residue was purified by column chromatography on silica gel, giving 2.2 g of the contemplated compound in amorphous form in 81 % yield.

Infrared absorption spectrum (KBr)

ν max (cm^{-1}): 1798, 1723

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.42 (3H, t), 1.48 (3H, s), 3.52-3.65 (2H, m), 4.36 (2H, q), 4.60-4.78 (2H, m), 5.10 (2H, s), 5.73 (1H, d), 5.90 (1H, d), 8.31 (1H, s)

Example 7

Preparation of iodomethyl 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 10)

A 1.73 g quantity of chloromethyl 2ß-(4-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-

3α-carboxylic acid-1,1-dioxide and 1.3 g of sodium iodide were stirred in 3.4 ml of acetone at room temperature for 18 hours. To the reaction mixture was added 2.9 ml of water and the pH of the resulting mixture was adjusted to 7 to 8 with an aqueous solution of sodium hydrogen-carbonate. After addition of 2.9 ml of water, the mixture was decolorized with an aqueous solution of 0.5 M sodium thiosulfate, extracted with dichloro-methane, washed with water and dried over magnesium sulfate. The solvent was removed by distillation and 1.9 g of the contemplated compound was prepared in amorphous form in 90 % yeild.

Infrared absorption spectrum (KBr)

ν max (cm^{-1}): 1798, 1725

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.42 (3H, t), 1.49 (3H, s), 3.52-3.68 (2H, m), 4.43 (2H, q), 4.59-4.78 (2H, m), 5.09 (2H, s), 5.96 (1H, d), 6.07 (1H, d), 8.32 (1H, s)

Example 8

Preparation of sodium 2ß-(5-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 11)

A 220 mg of the contemplated compound was

prepared in the form of white powder in the same manner as in Example 4 from 0.34 g of p-nitrobenzyl 2ß-(5-ethoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide in 83 % yield.

The white powder thus obtained decomposed at a temperature of over 180°C.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1788, 1736

Nuclear magnetic resonance spectrum (D_2O)

δ (ppm) : 1.39 (3H, t), 1.43 (3H, s), 3.40 (1H, dd), 3.71 (1H, dd), 4.46 (2H, q), 4.57 (1H, s), 4.96-5.05 (1H, m), 5.40 (1H, d), 5.82 (1H, d), 8.34 (1H, s)

Example 9

Preparation of sodium 2ß-(4-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 12)

A 0.18 g quantity of the contemplated product was prepared as white powder in the same manner as in Example 4 from 0.3 g of p-nitrobenzyl 2ß-(4-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide in 78 % yield.

The white powder thus obtained decomposed at a temperature of over 184°C.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1782, 1730

Nuclear magnetic resonance spectrum (D_2O)

δ (ppm) : 1.46 (3H, s), 3.45 (1H, dd), 3.73 (1H, dd), 3.97 (3H, s), 4.50 (1H, s), 4.81 (2H, s), 4.98-5.10 (1H, m), 5.18 (1H, d), 5.42 (1H, d), 8.72 (1H, s)

Example 10

Preparation of sodium 2ß-(5-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 13)

A 0.19 g quantity of the contemplated compound was prepared as white powder in the same manner as in Example 4 from 0.3 g of p-nitrobenzyl 2ß-(5-methoxycarbonyl-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide in 82 % yield.

The white powder thus obtained decomposed at a temperature of over 180°C.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1778, 1730

Nuclear magnetic resonance spectrum (D_2O)

δ (ppm) : 1.41 (3H, s), 3.41 (1H, dd), 3.71 (1H, dd), 3.98 (3H, s), 4.56 (1H, s), 4.95-5.08 (1H, m), 5.40 (1H, d), 5.83 (1H, d), 8.34 (1H, s)

Example 11

Proparation of p-nitrobenzyl 2α-methyl-2ß-[4-(p-nitrobenzyloxycarbonyl)-1,2,3-triazol-1-yl]methylpenam-3α-carboxylate-1,1-dioxide (Compound 14) and p-nitrobenzyl 2α-methyl-2ß-[5-(p-nitrobenzyloxycarbonyl)-1,2,3-triazol-1-yl]methylpenam-3α-carboxylate-1,1-dioxide (Compound 15)

A 4 g quantity of p-nitrobenzyl 2ß-adidomethyl-2α-methylpenam-3α-carboxylate-1,1-dioxide and 8.2 g of p-nitrobenzyl acetylene carboxylate in 100 ml of benzene were refluxed under nitrogen atmosphere for 12 hours. The solvent was distilled off at reduced pressure. The residue was subjected to column chromatography on silica gel to provide 3.6 g of p-nitrobenzyl 2α-methyl-2ß-[4-(p-nitrobenzyloxycarbonyl)-1,2,3-triazol-1-yl]-methylpenam-3α-carboxylate-1,1-dioxide (Compound 14) and 0.9 g of p-nitrobenzyl 2α-methyl-2ß-[5-(p-nitrobenzyloxycarbonyl)-1,2,3-triazol-1-yl]methylpenam-3α-carboxylate-1,1-dioxide (Compound 15) both in amorphous form.

Compound 14

Infrared absorption spectrum (KBr)

$\nu max\ (cm^{-1})$: 1800, 1740

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.34 (3H, s), 3.3-3.8 (2H, m), 4.67 (1H, s), 4.60-4.76 (1H, m) 5.12 (2H, s), 5.37 (2H, s), 5.48 (2H, s), 7.5-7.7 (4H, m), 8.1-8.3 (4H, m), 8.37 (1H, s).

Compound 15

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1800, 1740

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.41 (3H, s), 3.3-3.7 (2H, m), 4.6-4.7 (1H, m), 5.07 (1H, s), 5.1-5.6 (4H, m), 5.46 (2H, s), 7.4-7.7 (4H, m), 8.15 (1H, s), 8.1-8.4 (4H, m)

<u>Example 12</u>

Preparation of dipotassium 2ß-(4-carboxy-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 16)

Hydrogenation was conducted in 100 ml of ethyl acetate and 100 ml of water at room temperature for 1 hour by using 3.6 g of p-nitrobenzyl 2α-methyl-2ß-[4-(p-nitrobenzyloxycarbonyl)-1,2,3-triazol-1-yl)]-methylpenam-3α-carboxylate-1,1-dioxide, 2.0 g sodium hydrogencarbonate and 0.68 g of 10 % palladium charcoal,

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catalyst. Thereafter the aqueous layer was separated and was washed once with ethyl acetate, and the pH thereof was adjusted to 1.5 to 1.7 with 6 N hydrochloric acid. The aqueous solution was saturated with sodium chloride and extracted a few times with ethyl acetate. The ethyl acetate solutions thus formed were collected and dried over magnesium sulfate. The solvent was distilled off at reduced pressure to provide as the residue a foamed product of 2ß-(4-carboxy-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylic acid-1,1-dioxide.

A 2 g quantity of the 2ß-(4-carboxy-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylic acid-1,1-dioxide was dissolved in 20 ml of butanol. To the solution was added a solution of potassium 2-ethyl hexanoate in butanol, and the mixture was stirred awhile at room temperature. The precipitate was filtered to give 2.0 g of white solids having a melting point of over 178°C (decomposition).

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1780, 1610

Nuclear magnetic resonance spectrum (D_2O)

δ (ppm) : 1.47 (3H, s), 3.49 (1H, dd), 3.77 (1H, dd), 4.53 (1H, s), 5.0-5.1 (1H, m), 5.16 (1H, d), 5.41 (1H, d), 8.47 (1H, s)

Example 13

Preparation of dipotassium 2ß-(5-carboxy-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 17)

White solid of the contemplated compound with a melting point of over 175°C (decomposition) was prepared in the same manner as in Example 12 by using p-nitrobenzyl 2α-methyl-2ß-[5-(p-nitrobenzyloxycarbonyl)-1,2,3-triazol-1-yl]methylpenam-3α-carboxylate-1,1-dioxide.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1780, 1610

Nuclear magnetic resonance spectrum (D_2O)

δ (ppm) : 1.40 (3H, s), 3.43 (1H, dd), 3.71 (1H, dd), 4.58 (1H, s), 4.9-5.1 (1H, m), 5.36 (1H, d), 5.93 (1H, d), 8.04 (1H, s)

Example 14

Preparation of benzhydryl 2ß-(4-carboxy-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 18)

A 0.5 g quantity of benzhydryl 2ß-azidomethyl-2α-methylpenam-3α-carboxylate-1,1-dioxide and 0.083 g of acetylenecarboxylic acid were stirred in 2 ml of dichloromethane at room temperature under nitrogen atmosphere for 24 hours. The solvent was removed by distillation at

reduced pressure and to the residual oil was added benzene. The insolubles were filtered off and to the residue was added hexane to deposit crystals which were collected by filtration. Thus there was produced 0.23 g of white crystals which melt at 120 to 121°C.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1805, 1745

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.07 (3H, s), 3.2-3.8 (2H, m), 4.5-4.7 (1H, m), 4.69 (1H, s), 5.12 (2H, bs), 7.02 (1H, s), 7.1-7.6 (10H, m), 8.33 (1H, s)

Example 15

Preparation of disodium 2ß-(4-carboxly-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide (Compound 19)

Hydrogenation was conducted in 10 ml of ethyl acetate and 10 ml of water at room temperature for 30 minutes by using 49 mg of benzhydryl 2ß-(4-carboxly-1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylate-1,1-dioxide, 15 ml of 10 % palladium charcoal and 24 mg of sodium hydrogencarbonate. The aqueous layer was separated from the reaction mixture and washed with ethyl acetate, and was purified with an MCI gel, CHP-20P (product of Mitsubishi Kasei Co., Ltd., Japan).

After freeze-drying, there was obtained a white amorphous product having a melting point of 220 to 250°C (decomposition).

The values of the infrared absorption spectrum and nuclear magnetic resonance spectrum of the compound thus obtained were similar to those of Compound 16 prepared in Example 12.

Example 16

Preparation of benzhydryl 2α-methyl-2ß-(4-trimethylsilyl-1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide (Compound 20)

A 150 mg quantity of benzhydryl 2ß-azidomethyl-2α-methylpenam-3α-carboxylate-1,1-dioxide was reacted in a sealed reactor with 300 mg of trimethylsilylacetylene at 90 to 95°C for 20 hours. The reaction mixture was concentrated at reduced pressure, giving 170 mg of white crystals which melt at 172 to 175°C.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1805, 1755

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 0.32 (9H, s), 1.05 (3H, s), 3.3-3.7 (2H, m), 4.5-4.7 (1H, m), 4.65 (1H, s), 5.08 (2H, AB-q), 7.00 (1H, s), 7.3-7.5 (10H, m), 7.67 (1H, s)

Example 17

Preparation of benzhydryl 2α-methyl-2β-(1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide (Compound 21)

A 133 mg quantity of benzhydryl 2α-methyl-2β-(4-trimethylsilyl-1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide, 3.26 g of 18-crown-6(1,4,7,10,13,16-hexaoxacyclooctadecane) and 15.8 mg of potassium fluoride were stirred in 0.7 ml of N,N-dimethylformamide at 50 to 60°C for 5.5 hours. The reaction mixture was poured into excess iced water and the mixture was extracted a few times with ethyl acetate. The ethyl acetate extracts were collected and dried over magnesium sulfate. The solvent was distilled off at reduced pressure and the residue was purified by column chromatography on silica gel, whereby a white proudct was given which has a melting point of 206 to 208°C (decomposition).

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1800, 1760

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.05 (3H, s), 3.3-3.7 (2H, m),
4.5-4.7 (1H, m), 4.65 (1H, s),
5.10 (2H, AB-q), 7.00 (1H, s),
7.3-7.5 (10H, m), 7.73 (1H, s)

Example 18

Preparation of benzhydryl 2α-methyl-2β-(1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide (Compound 21)

A 500 mg quantity of benzhydryl 2β-azidomethyl-2α-methylpenam-3α-carboxylate-1,1-dioxide, 335 mg of trimethylsilylacetylene and 2 ml of methylene chloride were reacted in a sealed reactor at 95°C for 20 hours. The reaction mixture was concentrated at reduced pressure and the residue was purified by column chromatography on silica gel to provide white solids having a melting point of 203 to 204°C (decomposition).

Fast atomic bombardment mass spectrum method;

$m/e=467(M^+)$

The values of the infrared absorption spectrum and nuclear magnetic resonance spectrum of te compound thus obtained were identical with those of Compound 21 obtained in Example 17.

Example 19

Preparation of benzhydryl 2α-methyl-2β-(1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide (Compound 21)

A 200 mg quantity of benzhydryl 2β-azidomethyl-2α-methylpenam-3α-carboxylate-1,1-dioxide was reacted with 10 ml of vinyl acetate in a sealed reactor at 100 to 110°C for 30 hours. The reaction mixture was concentrated at reduced pressure. The residue was crystallized with cooled chloroform.

The white crystals thus obtained were found to have a melting point (decomposition) and the values of the nuclear magnetic resonance spectrum which were all identical with the values of Compound 21 obtained in Example 17.

Example 20

Preparation of sodium 2α-methyl-2ß-(1,2,3-triazol-1-yl)-methylpenam-3α-carboxylate-1,1-dioxide (Compound 22)

Hydrogenation was conducted in 10 ml of ethyl acetate and 10 ml of water at room temperature for 30 minutes by using 45 mg of benzhydryl 2α-methyl-2ß-(1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide, 15 mg of 10 % palladium charcoal and 16 mg of sodium hydrogencarbonate. The aqueous layer was separated from the reaction mixture and washed once with ethyl acetate. The aqueous solution was then purified with an MCI gel, CHP-20P (product of Mitsubishi Kasei Co., Ltd., Japan). After freeze-drying, there was obtained an amorphous product with a melting point of over 170°C (decomposition).

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1780, 1630

Nuclear magnetic resonance spectrum (D_2O)

δ (ppm) : 1.41 (3H, s), 3.45 (1H, dd), 3.72 (1H, dd), 4.48 (1H, s),

4.96-5.10 (1H, m),
5.25 (2H, AB-q), 7.85 (1H, d),
8.13 (1H, d)

Example 21

Preparation of p-nitrobenzyl 2α-methyl-2β-(1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide (Compound 23)

A 1.02 g quantity of p-nitrobenzyl 2β-azidomethyl-2α-methylpenam-3α-carboxylate-1,1-dioxide was reacted with 50 ml of vinyl acetate in a sealed reactor at 100 to 110°C for 30 hours. The reaction mixture was concentrated at reduced pressure and the residue was purified by column chromatography on silica gel, giving 0.73 g of the contemplated compound in amorphous form in 67 % yield which melts at 182 to 184°C.

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1800, 1760

Nuclear magnetic resonance spectrum ($CDCl_3$)

δ (ppm) : 1.26 (3H, s), 3.5-3.6 (2H, m),
4.66 (1H, s), 4.6-4.7 (1H, m)
5.07 (2H, s), 5.36 (2H, s),
7.61 (2H, d), 7.74 (1H, d),
7.80 (1H, d), 8.28 (2H, d)

Example 22

Preparation of sodium 2α-methyl-2ß-(4-trimethylsilyl-1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide (Compound 24)

Hydrogenation was performed in 15 ml of ethyl acetate and 15 ml of water at room temperature for 30 minutes by using 200 mg of benzhydryl 2α-methyl-2ß-(4-trimethylsilyl-1,2,3-triazol-1-yl)methylpenam-3α-carboxylate-1,1-dioxide, 50 mg of 10 % palladium charcoal and 98 mg of sodium hydrogencarbonate. The aqueous layer was removed from the reaction mixture and washed once with ethyl acetate. The aqueous solution was purified with an MCI gel, CHP-20P (product of Mitsubishi Kasei Co., Ltd., Japan). After freeze-drying, there was obtained an amorphous product having a melting point of over 170°C (decomposition).

Infrared absorption spectrum (KBr)

νmax (cm^{-1}): 1780, 1630

Nuclear magnetic resonance spectrum (D_2O)

δ (ppm) : 0.32 (9H, s), 1.38 (3H, s), 3.1-3.7 (2H, m), 4.46 (1H, s), 4.9-5.0 (1H, m), 5.23 (2H, AB-q), 8.16 (1H, s)

P The compounds obtained in some of the examples were checked for ß-lactamase inhibitory activity and antibacterial activity.

P1 (1) Test for ß-lactamase inhibitory activity

P The inhibitory activity against penicillinase (ß-lactamase) from Bacillus SP was measured by micro-iodometry Tanpakushitsu Kakusan Koso (Protein Nucleic Acid Enzyme), vol. 23, No.5, pp 391-400 (1978) using a penicillin G as a substrate. Table 1 given below shows the results.

Table 1

T510X

Compound	50 % Inhibitory Concentration
Compound 7	5.4×10^{-8}M
" 11	3.4×10^{-7}M
" 12	4.9×10^{-8}M
" 13	3.0×10^{-7}M
" 16	6.0×10^{-7}M
" 17	1.7×10^{-6}M
" 22	6.9×10^{-7}M
" 24	5.1×10^{-7}M

P1 (2) Test for antibacterial activity

1) Effects by ampicillin as combined with the present compound

P The compounds of the present invention and ampicillin, each singly used, were checked for minimal

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inhibitory concentration (MIC) against the bacteria listed in Table 2 given below by micro-broth dilution method ("American Journal Clinical Pathology" published in 1980, vol. 73, No.3, pp 374 to 379). The MIC of ampicillin as combined with the present compound (10 μg/ml) was measured against the same bacteria. In the method, the bacteria cultivated in Mueller Hinton Broth (product of DIFCO) and diluted to 10^7 CFU/ml were inoculated into the same medium containing ampicillin and the present compound in a specific concentration, and incubated at 37°C for 20 hours. Thereafter the growth of the microorganisms was observed to determine the minimal inhibitory concentration (MIC) for rendering the inoculated medium free from turbidity. The present compounds, singly used, turned out to be all more than 25 μg/ml in MIC. The bacteria as used in the test were those capable of producing ß-lactamase, among which the bacteria marked * in the table are those collected from the living body of human hosts and the others are a stock culture.

In Table 2, the present compounds are shown by the compound number.

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T530X

Table 2

Test Bacteria	MIC (μg/ml)								
	Ampicillin (singly used)	Present Compound (combined with ampicillin)							
		7	11	12	13	16	17	22	24
S.aureus S-54	25	0.1	0.2	0.2	0.2	0.2	0.78	0.2	0.78
S.aureus ATCC 90124	25	0.1	0.2	0.2	0.2	0.2	0.78	0.1	0.39
E.coli TH-13*	400	6.25	25	3.13	6.25	6.25	0.05	3.13	100
E.coli TH-397*	400	6.25	12.5	3.13	6.25	3.13	6.25	6.25	50
P.mirabilis 121	400	1.56	0.78	0.78	0.78	0.78	0.39	0.78	25
P.vulgaris IID OX-19	100	0.78	0.78	0.39	0.39	1.56	1.56	0.78	1.56
S. marcescens TH-05*	400	12.5	25	12.5	25	6.25	1.56	3.13	100

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(2) Effects by antibiotics as combined with the present compound

The compounds of the present invention, ampicillin, mecillinam, piperacillin and cephalexin, each singly used, were also tested for minimal inhibitory concentration against 30 strains of coliform bacilli collected from the living body of humans. The MIC of each antibiotic as combined with the present compound (10 μg/ml) was likewise measured. Table 3 to 6 indicate the results in which MIC_{50} and MIC_{70} indicate the minimal inhibitory concentration for inhibiting the growth of 50 % and 70 % respectively of the strains. The MICs of the present compounds singly used were all more than 25 μg/ml.

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T550X

Table 3

30 Strains of coriform bacilli	Ampicillin singly used	Present compound as combined with ampicillin				
		Comp. 7	Comp. 11	Comp. 16	Comp. 17	Comp. 22
MIC_{50} (μg/ml)	400	6.25	50	6.25	25	3.13
MIC_{70} (μg/ml)	400	50	100	6.25	100	6.25

T551X

Table 4

30 Strains of coriform bacilli	Mecillinam singly used	Present compound as combined with mecillinam				
		Comp. 7	Comp. 11	Comp. 16	Comp. 17	Comp. 22
MIC_{50} (μg/ml)	3.13	0.2	0.2	0.1	0.05	0.1
MIC_{70} (μg/ml)	12.5	0.39	0.39	0.1	0.39	0.2

T360X

Table 5

30 Strains of coriform bacilli	Piperacillin singly used	Present compound as combined with piperacillin				
		Comp. 7	Comp. 11	Comp. 16	Comp. 17	Comp. 22
MIC_{50} (μg/ml)	50	1.56	6.25	1.56	6.25	1.56
MIC_{70} (μg/ml)	200	6.25	25	3.13	50	1.56

T361X

Table 6

30 Strains of coriform bacilli	Cephalexin singly used	Present compound as combined with cephalexin				
		Comp. 7	Comp. 11	Comp. 16	Comp. 17	Comp. 22
MIC_{50} (μg/ml)	25	12.5	12.5	6.25	3.13	12.5
MIC_{70} (μg/ml)	100	100	100	25	12.5	50

Given below are examples of preparation of the present antibacterial compositions.

Preparation Example 1

T570X

Ampicillin	200 mg
Compound 22	200 mg
Lactose	100 mg
Crystalline cellulose	57 mg
Magnesium stearate	3 mg
Total	560 mg

(amount per capsule)

The above ingredients are formulated in the proportions listed above into a capsule.

Preparation Example 2

T571X

Amoxycillin	100 mg
Compound 16	70 mg
Lactose	330 mg
Corn starch	490 mg
Hydroxypropyl methyl cellulose	10 mg
Total	1000 mg

(amount per dose)

The above ingredients are formulated in the proportions listed above into granules.

Preparation Example 3

T572X

Pivmecillinam	70 mg
Compound 17	70 mg

Lactose	33 mg
Crystalline cellulose	15 mg
Magnesium stearate	3 mg
Talc	4 mg
Corn starch	15 mg
Hydroxypropyl methyl cellulose	10 mg
Total	220 mg
	(amount per tablet)

P The above ingredients are formulated in the proportions listed above into a tablet.

Preparation Example 4

T580X

Compound 22	120 mg
Hydroxypropyl cellulose	3 mg
Corn starch	25 mg
Magnesium stearate	2 mg
Total	150 mg
	(amount per tablet)

P The above ingredients are formulated in the proportions listed above into a tablet.

5X